J. Glob. Innov. Agric. Sci., 2022, 10(1):19-24.
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/10.972
http://www.jgiass.com

Assessment of mutation caused by colchicine in apple gourd (*Praecitrullus fistulosus*) based on morphological and biochemical attributes

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Apple gourd is a very important vegetable. It has problem of more seeds which increase the dislikeness of customers in the market. Research and development are required to reduce the number of seeds for more consumer acceptability. Colchicine may be used as one of the most potential treatments in the propagation which is used to induce mutation and prevention of microtubule formation during cell division and doubling of chromosomes. In this study Colchicine was used. The seed of Apple gourd was treated with two different concentrations T^1 and T^2 in comparison with control T^0. Treated seeds were sown in polyethene bags in nursery for germination and after 40 days were transplanted in the field. This field experimentation was carried out in a research area of department of horticulture sciences according to complete randomized block design (CRBD). The following parameters were recorded (number of leaves per plant, number of branches per plant, number of flowers per plant, flowering time, number of fruits per plant, fruit yield per plant, fruiting time, fruit weight (g), fruit diameter (mm), leaf area (cm2), vine length (cm) and germination %, leaf area, total proteins, total phenolics and stomata contents, carbohydrates contents, chlorophyll A and chlorophyll B contents). Morphological parameters number of leaves per plant, number of branches per plant, number of flowers per plant, flowering time, number of fruits per plant, fruit yield per plant, fruiting time, fruit weight (g), fruit diameter (mm), leaf area (cm2), vine length (cm) and germination % were increased in (T^0). While large number of leaves, thicker branches, broad and large leaves, dark flowers and tasteful and seedless fruits were produced in 0.2% colchicine solution (T^2). On the other hand, biochemical or physiological parameters leaf area, total proteins, total phenolics and stomata contents were increased in (T^0), carbohydrates contents were increased in (T^1), chlorophyll A and chlorophyll B contents were increased in (T^2). Overall colchicine had good effect on chromosomes number, morphological and physiological attributes of apple gourd. According to present study 0.2 % of colchicine solution is considered best for the production of tetraploid plants and to induce polyploidy in vegetables.

Keywords*:* Polyploidy, colchicine, apple gourd, Genetic diversity, morphological parameters and biochemical parameters.

INTRODUCTION

Apple gourd (*Praecitrullus fistulosus*) commonly known as 'tinda' is an important and popular annual summer vegetable. Its botanical name is *Praecitrullus fistulosus*. It belongs to the family cucurbitaceae and its chromosomes number (2n) is 24 (Kirtikar, 1998). It is native to India and Pakistan (Schippers, 1994). It has a pleasant flavor and it is widely consumed in various parts of the world (Kirtikar, 1998). Its seeds are utilized as fodder for animals (Chadha and Kallo, 1993). The mature fruits of tinda are used to produce to produce pickles in India and Pakistan (Grubben *et al.*, 2004). While immature fruits are used to make rayata, vegetable curries and other dishes. Also, the leaves are used for medicinal purpose (Major, 2017). In Agriculture colchicine is a chromosomal doubling agent that can be used to induce polyploidy in a variety of crops (Kazi, 2015). In Horticulture sciences colchicine have been used for mutation to develop new strains of different crops including watermelon (Wood, 1955) and pointed gourd (Khaira, 1951). According to the information available in literature colchicine has now been tested in Apple gourd. It is hypothesized that the application of colchicine will create variation in morphological, physiological and biochemical attributes of Apple gourd through mutation. Keeping in view importance of colchicine for production of diploids and tetraploids in cucurbits, an experiment had been conducted to study an impact related to different colchicine concentration upon following attributes of apple gourd

Rasheed, N., I. Ahmad, M. Nafees and M.I. Hassan. 2022. Assessment of mutation caused by colchicine in apple gourd (*praecitrullus fistulosus*) based on morphological and biochemical attributes. Journal of Global Innovations in Agricultural Sciences. 10:19-24.
[Received July 2022; Accepted 14 Nov 2021; Published 28 Mar 2022]

MATERIALS AND METHODS

The research was conducted in the research area of the Department of Horticultural Sciences, Islamia University of Bahawalpur. The trial included 2 treatments one control, 0.1%, and 0.2 % field capacity. Each treatment had three replications, each contained 40 seeds. Seeds were soaked in 0.1 and 0.2 % colchicine solution for 48 hours before planting. In this experiment, 360 colchicine treated seeds were planted in polyethene bags at horticulture nursery. Regular cultural treatments like as watering and weeding were carried out throughout the growing season. After 65 days seedlings were transplanted in field. Two different concentrations of colchicine were used for seed treatment of apple gourd: 0.1%, and 0.2%. The experiment was set up using a Completely Randomized Block Design (CRBD) design. The analysis of variance (ANOVA) methodology was used to evaluate all of the data was used to compare the treatments' means and statistical significance was established using the least significant difference test (LSD) within a 5% probability level. (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

***Number of flowers per plant*:** The effect of colchicine was statistically highly significant ($p<0.00$) on number of flowers bloom per plant according to the analysis of variance (ANOVA) Table 1. The results showed that maximum number of flowers bloom per plant (75.66) were recorded in T^0 (control) followed by the T^1 (0.1% C) and T^2 (0.2% C). Both treatments showed 6.66 and 49 flowers per plant respectively. Overall mean of colchicine showed that maximum number of flowers bloom (6.667) was recorded in T^1 (0.1%) whereas minimum number of flowers was recorded (49) in T^2 (0.2%) colchicine Treatment. Similar results were obtained from 0.1 % of colchicine solution in research in which they investigated that the tetraploid seedlings treated with colchicine had more flowers per plant (Suliman and Asander (2020).

Table 1. Analysis of variance (ANOVA) for number of flowers of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	59.111		
Treat	2	536.111	79.10	0.0006
Error	4	6.778		
Total	8			

Grand Mean 61.778 CV 4.21

***Flowering time (days*):** The effect of colchicine was statistically significant ($p<0.05$) on the emergence of flowering according to the analysis of variance (ANOVA) Table 2. The results showed that maximum days to flowers emergence (42.33) were recorded in T^1 (0.1% C) followed by the T2 (0.2% C) and T^0 (control). Overall mean of colchicine showed that maximum number of branches (42.33) was recorded in T^1 (0.1%) whereas minimum number of days to flowerings emergence was recorded (45) in T^2 control Treatment. Similar results obtained from flower buds of Cape gooseberry (*Physalis peruviana L.*) treated with colchicine at a rate of 0.10 percent for 12 hours using the cotton plug technique took longer to emerge than control flower buds. This discovery proved the existence of genetic diversity in the response of Cape gooseberry plant growth and flowering features to ploidy alteration (*Physalis peruviana L.*) (Ravindra *et al.* (2019).

Table 2. Analysis of variance (ANOVA) for number of flowering times of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	0.778		
Treat	2	80.444	10.34	0.0263
Error	4	7.778		
Total	8			

Grand Mean 40.778 CV 6.84

***Number of fruits per plant*:** The effect of colchicine was statistically highly significant ($p<0.00$) on number of fruits according to the analysis of variance (ANOVA) Table 3. The results showed that maximum number of fruits (12) was recorded in T0 (control) followed by the T^1 (0.1% C) and T^2 (0.2% C). Both treatments showed 7.6 and 5 number of fruits per plant respectively. Overall mean of colchicine showed that maximum number of branches (7.6) was recorded in T^1 control treatment whereas minimum number of fruits was recorded (5) in T^2 (0.2%) colchicine Treatment. Similar results were obtained in colchicine-induced autotetraploids of red-fleshed kiwifruit (*Actinidia chinensis Planch.*). Similar results were obtained in UC-82B variety of tomato responds to colchicine and enviromental fluctuations for better growth and improvement of yield (Aminu *et al.* ((2021).

Table 3. Analysis of variance (ANOVA) for number of fruits of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	11.111		
Treat	2	37.444	59.13	0.0011
Error	4	1.111		
Total	8			

Grand Mean 8.2222 CV 12.82

***Fruiting time (days*):** The effect of colchicine was statistically highly significant ($p<0.00$) on the days of fruiting emergence according to the analysis of variance (ANOVA) Table 4. The results showed that maximum days to fruits emergence (81) were recorded in T^0 (Control) followed by the T^1 (0.1% C) and T^2 (0.2% C). Overall mean of colchicine showed that maximum number of branches (66) was recorded

in T^1 (0.1%) whereas minimum number of days to fruits emergence was recorded (55) in T^2 control Treatment. Similar results were obtained in research to study pollen effects on fruit attributes and seed properties in colchicine-induced autotetraploids of red-fleshed kiwifruit (*Actinidia chinensis Planch*.) (*Wu et al*. (2014).

Table 4. Analysis of variance (ANOVA) for fruiting time of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	76.000		
Treat	2	522.333	59.13	0.0011
Error	4	8.833		
Total	8			

Grand Mean 67.333 CV 4.41

***Vine length (cm)*:** The effect of colchicine was statistically highly significant (p<0.00) on the length of vine per plant according to the analysis of variance (ANOVA) Table 5. The maximum vine length (313.76cm) was achieved in T^0 followed by T^2 (0.2%C) and T^1 (0.1%C) respectively. Overall mean of colchicine showed maximum length of vine (104.79cm) was attained in T^2 minimum length was attained in T^1 respectively. Similar results were obtained in research to study the Sensitivity of some quantitative and yield characters of 'Egusi' melon (*Colocynthis citrullus L*.) to treatment with microtubule inhibitors. Seeds treated with various concentrations of oryzalin and colchicine had no significant influence (P>0.05) on vine length, possibly indicating that this parameter was not severely altered by treatment (Ebiamadon *et al.* (2011).

Table 5. Analysis of variance (ANOVA) for vine length (cm) of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	1.0		
Treat	2	48731.3	1521.39	0.0000
Error	4	32.0		
Total	8			

Grand Mean 167.13 CV 3.39

***Germination %*:** The effect of colchicine was statistically highly significant (p<0.00) on the percentage of germination per plant according to the analysis of variance (ANOVA) Table 6. The results showed the maximum percentage (37.33%) of germination T^0 followed by T^2 (0.2%C) and T^1 (0.1%C). Overall mean of colchicine showed maximum percentage of germination (32.33) was attained in T^2 minimum length (21.33) was attained in T^1 respectively. Similar results were obtained in Capsicum annum, Sodium Azide treatment induces high germination percentages (Ulmalkar *et al.* (1998).

***Determination of leaf area*:** The effect of colchicine was statistically highly significant (p<0.00) on the determination of leaf area per plant according to the analysis of variance (ANOVA) Table 7. The results showed the maximum (104.07) determination of leaf area in T^0 followed by T^2 (0.2%C) and T^1 (0.1%C). Both treatments showed 66.87 and 27.33 leaf area per plant respectively. Overall mean of colchicine showed maximum leaf area (66.87) was attained in T^2 and minimum leaf area was attained in T^1 respectively. In present study results were contradictory according to (Hoballah (1999) who reported colchicine-induced increases in leaf area in false sesame mutants are consistent with the findings of Maluszynski *et al.* (2001) and (Pasztor *et al.* (1985) they reported an increase in leaf area among Zea mays mutagens.

Table 6. Analysis of variance (ANOVA) for germination % of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	4.333		
Treat	2	201.000	41.59	0.0021
Error	4	4.833		
Total	8			

Grand Mean 30.333 CV 7.25

Table 7. Analysis of variance (ANOVA) for leaf area of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	14.10		
Treat	2	4417.36	8728.05	0.0000
Error	4	0.51		
Total	8			

Grand Mean 66.089 CV 1.08

***Determination of stomata contents*:** The effect of colchicine was statistically highly significant (p<0.00) on the determination of stomata contents per plant according to the analysis of variance (ANOVA) Table 8. The results showed the maximum (206.3) determination of stomata contents in T^0 followed by T^1 (0.1%C) and T^2 (0.2%C). Both treatments showed 111 and 40.2 number of stomata contents per leaf respectively. Overall mean of colchicine showed maximum determination stomata contents (111) per leaf was attained in T^1 and minimum determination of stomata contents (40.2) achieved was attained in T^1 respectively. Similar results were obtained to distinguish diploid and polyploid regenerates in Stevia rebaudiana Silva *et al*. (2000) and (Zhang *et al*. (2018).

Table 8. Analysis of variance (ANOVA) for stomata contents of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	15.4		
Treat	2	20842.0	14423.5	0.0000
Error	4	1.4		
Total	8			

Grand Mean 119.17 CV 1.01

***Determination of chlorophyll A contents*:** The effect of colchicine was statistically highly significant ($p<0.00$) on the determination of chlorophyll A contents per leaf according to the analysis of variance (ANOVA) Table 9. The results showed the maximum (1.2357) determination of chlorophyll A contents in T^2 followed by T^0 (Control) and T^1 (0.1%C). Both treatments showed 0.72 and 0.08 number of chlorophyll A contents per leaf respectively. Overall mean of colchicine showed maximum determination chlorophyll A contents (1.23) per leaf was attained in T^2 and minimum determination of chlorophyll A contents (0.08) achieved was attained in T^1 respectively. Similar results were obtained in research to investigate the assessment of biochemical attributes of *Praecitrullus fistulosus* treated with mutants. Chemical mutagens such as colchicine and ethidium bromide were utilized (Khan (2015).

Table 9. Analysis of variance (ANOVA) for chlorophyll A contents of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	0.00033		0.0000
Treat	2	1.00479	1673.69	
Error	4	0.00060		
Total	8			

Grand Mean 0.6811 CV 3.60

***Determination of total carbohydrates contents*:** The effect of colchicine was statistically significant ($p<0.05$) on the determination of carbohydrates contents per leaf according to the analysis of variance (ANOVA) Table 10. The results showed the maximum (174) determination of carbohydrates contents in T^1 (0.1%C) followed by T^0 (Control) and T^2 (0.2%C). Both treatments showed 170 and 158.7 number of carbohydrates content respectively. Overall mean of colchicine showed maximum determination carbohydrates contents (174) per leaf was attained in T^1 and minimum determination of carbohydrates contents (158.7) was attained in T^1 respectively. Similar results were obtained in research to investigate the assessment of biochemical attributes of *Praecitrullus fistulosus* treated with mutants. Chemical mutagens such as colchicine and ethidium bromide were utilized (Khan (2015).

Table 10. Analysis of variance (ANOVA) for carbohydrates contents of *praecitrullus fistulosus.*

Source	DF	MS	F	P
Rep	2	85.444		
Treat	2	189.778	10.88	0.0241
Error	4	17.444		
Total	8			

Grand Mean 167.56 CV 2.49

***Determination of total phenolics compounds*:** The effect of colchicine was statistically highly significant ($p<0.00$) on the determination of total phenolic compounds per leaf according to the analysis of variance (ANOVA) Table 11. The results showed the maximum (122.4) determination of total phenolics compounds in T^0 (Control) followed by T^2 (0.2%C) and T^1 (0.1%C). Both treatments showed 94.77 and 75.1 number of total phenolics compounds respectively. Overall mean of colchicine showed maximum (94.77) determination of total phenolics compounds was attained in T^2 and minimum determination of total phenolics compounds (75.1) was attained in T^1 respectively. Similar results were obtained in research to investigate the assessment of biochemical attributes of *Praecitrullus fistulosus* treated with mutants. Chemical mutagens such as colchicine and ethidium bromide were utilized (Khan (2015).

Table 11. Analysis of variance (ANOVA) for phenolics compounds of *praecitrullus fistulosus.*

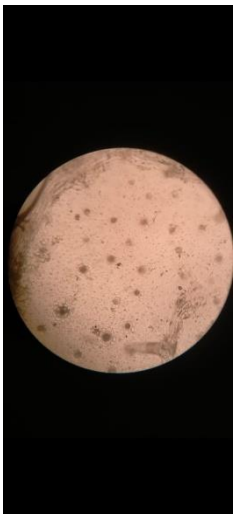
Source	DF	MS	F	P
Rep	2	10.14		
Treat	2	1693.83	277.17	0.0001
Error	4	6.11		
Total	8			

***Determination of total proteins*:** The effect of colchicine was statistically highly significant ($p<0.00$) on the determination of total proteins per leaf according to the analysis of variance (ANOVA) Table 12. The results showed the maximum (110.27) determination of total proteins in T^0 (Control) followed by T^2 (0.2%C) and T^1 (0.1%C). Both treatments showed 80.1 and 64.3 number of total proteins respectively. Overall mean of colchicine showed maximum determination of (80.1) total proteins attained in T^2 and minimum determination of total proteins (64.3) was attained in T^1 respectively. Similar results were obtained in research to investigate the assessment of biochemical attributes of *Praecitrullus fistulosus* treated with mutants. Chemical mutagens such as colchicine and ethidium bromide were utilized khan (2015).

Table 12. Analysis of variance (ANOVA) for proteins of *praecitrullus fistulosus.*

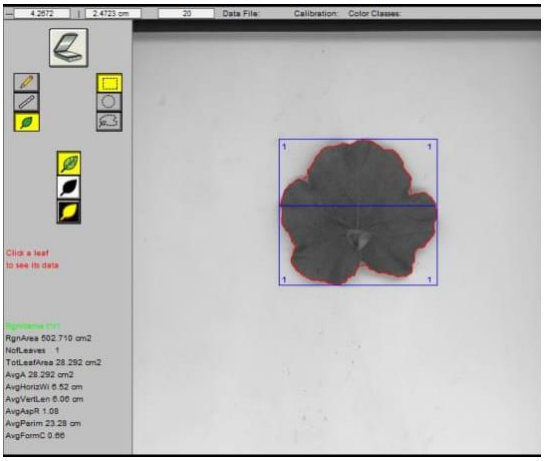
Source	DF	MS	F	P
Rep	2	3.95		
Treat	2	1636.30	559.53	0.0000
Error	4	2.92		
Total	8			

Grand Mean 84.889 CV 2.01

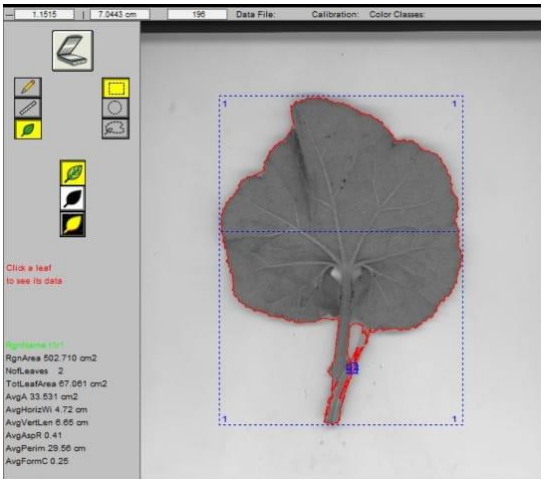


Stomata contents of control treatment (T^0)

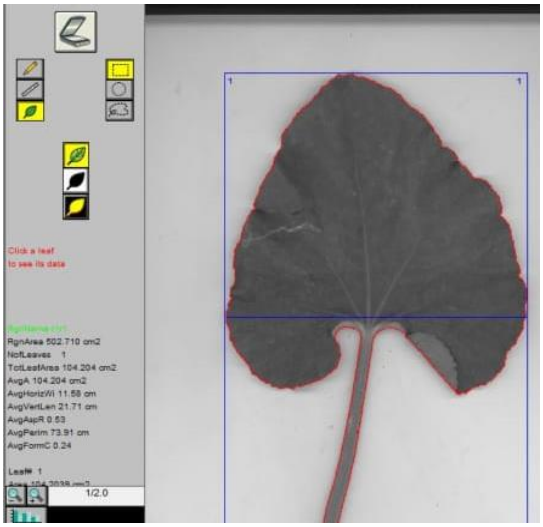
Stomata contents of 0.1% treatment (T^1)



Leaf analysis of 0.1% treatment (T^1)

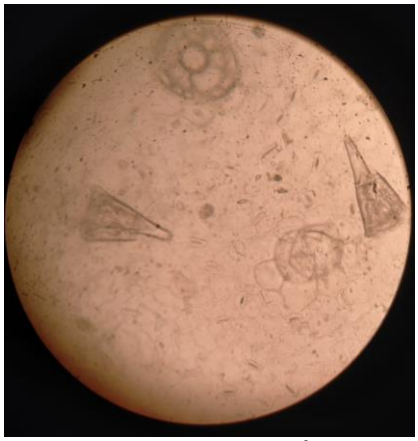


Leaf analysis of 0.2% treatment (T^2)



Stomata contents of 0.2% treatment (T^2)

Some pictures were taken during the experiment



Leaf analysis of control treatment (T^0)

***Conclusion*:** Colchicine is a best chemical to induce mutation and polyploidy in plants. Recent investigation shows that colchicine had a good effect on morphological and biochemical parameters of horticulture crops. It was found that 0.2% concentration of colchicine has the highest effect chlorophyll A, chlorophyll B and size of stomata. Colchicine at the rate of 0.2 % considered best to induce mutation in cucurbits. More investigation into the rate of colchicine, environmental conditions, and breeding methods can open new opportunities for the scientific community.

***Authors Contributions statement*:** In the current study Nazia Rasheed executed the experiment in the field, Ishtiaq Ahmad supervise the research work in terms of biochemical analysis and Muhammad Nafees and M. Intizar-ul- Hassan help in statistical analysis and write up improve.

***Conflict of interest*:** All authors have participated in conception and design, or analysis and interpretation of the data, drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

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